Manipulated Medicine

Genetic tricks turn stem cells into therapies

by Jenni Laidman

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It was the early 1990s and Evan Snyder was a young investigator invited to speak on the last afternoon of the last day of a genetics conference. The suitcases lined up along the back wall signaled the audience's preoccupation.

Snyder, M.D., Ph.D., today program director of the Sanford-Burnham Medical Research Institute in La Jolla, was representing the new-new thing: the notion that someday stem cells would play a role in gene therapy.

Even for Snyder, the idea of genetically engineering these newly discovered cells was purely theoretical. His work avoided the crowded field of genetics—a field awash in senior investigators. He stuck with the less-populated stem cell specialty, counting on the big fish/small pond equation to help his career.

Then his best friend was diagnosed with a deadly brain tumor. The children of Dr. James A. Galambos approached Snyder. Was there anything he could do? The question altered his course and he began a collaboration that included a new graduate student dedicated to using stem cells to tackle glioma.

In 2000, a paper in the *Proceedings of the National Academy of Science* bore a dedication to Snyder's late friend. It announced a finding that holds so much promise for the treatment of brain tumors that two grants from the California Institute for Regenerative Medicine are based on it, together totaling $37 million. Neural stem cells, it revealed, are attracted to tumors like moths to a light. If they could be made to carry a chemotherapeutic payload, they could serve as weapons against the wanton spread of metastatic brain cancer.

Snyder, senior author on that PNAS paper, is a co-investigator on one CIRM grant that intends to exploit this feature of stem cell behavior. Karen Aboody, the PNAS paper's first author, is the principal investigator on the other grant that will use neural stem cell's strange attraction to her advantage. Aboody, M.D., was the graduate student Snyder had added to his lab. Today she is an associate professor at City of Hope in Duarte.

Studies in mice using this stem-cell-based weapon against brain tumor have been remarkably successful. "When we tried it, 90 percent of the animals survived," Aboody said. Without the stem cell targeting, only 30 percent lived.

This attack on brain cancer is just one example of a new breed of therapy based on stem cells manipulated to become useful therapies. What Snyder presented as theory to that roomful of distracted conference attendees is now a field that's being mined for new treatments for glioma, HIV/AIDS, sickle cell anemia, heart disease and a rare skin disorder called epidermolysis bullosa, all of which are the focus of CIRM disease team awards. These teams are expected to bring their manipulated stem cells to the FDA to begin clinical trials within four years.

Genetic Resistance

Since the discovery of HIV/AIDS, researchers have puzzled over a handful of people who never contracted the virus despite repeated exposure. By the early 1990s scientists had identified a rare mutation as the protective agent.

To enter immune cells, HIV docks with two proteins on the cell's surface — a primary receptor known as CD4, and a secondary receptor called CCR5. HIV-resistant patients make an altered CCR5 protein that prevents the virus from docking. A team of German researchers put this knowledge to work and in 2009 published a paper in the New England Journal of Medicine describing a stem cell transplant given to a 40-year-old leukemia patient infected with HIV. The Berlin researchers used a matching donor who carried the mutated CCR5 genes. Today, the recipient of those cells, known as the Berlin patient, is considered functionally cured of HIV. He takes

no HIV medication, has no disease symptoms, and shows no apparent virus in his body.

This news of the German stem cell transplant fuels hope for these new stem-cell approaches, says Jeff Sheehy, communications director at the AIDS Research Institute at the University of California, San Francisco and CIRM governing board member.

“Until Berlin, there wasn’t much promise for HIV and stem cells,” Sheehy said.

The new stem-cell therapeutic approach comes at what has been a sobering moment in the history of AIDS treatment, Sheehy said.

Despite years of effort, there is still no vaccine against the disease on the horizon, and successful antiretroviral therapy, while saving many lives, is generally unavailable in the developing world, and remains enormously expensive. It comes with a growing list of side effects, including a rise in heart disease, an increase in non-HIV related cancers, such as lung cancer, and elevated cholesterol and triglyceride levels. “

"A person living with HIV, even with well-controlled virus, even with antiretroviral treatment, is looking at least a 10 year shorter life expectancy,” Sheehy said.

Despite the hope embodied by the Berlin patient, transplantation isn’t considered a widespread cure for HIV/AIDS according to John Zaia, M.D., chair of virology at City of Hope, who leads one of the two HIV/AIDS disease teams. Zaia’s team, and a second team lead by Irvin Chen, Ph.D., director of the AIDS Institute at UCLA, plan instead to genetically interfere with CCR5 in the blood-forming stem cells of HIV patients.

Although the details of the research plans differ, they are similar in their general outline: harvest a patient’s blood-forming stem cells, genetically alter the cells outside the body so they no longer produce the CCR5 protein, and return the HIV-resistant cells to the patient where they will reform the patient’s entire blood system.

A related disease team project, led by Donald Kohn, M.D. at UCLA, will also modify blood-forming stem cells, this time to treat people with sickle cell anemia. The disease is caused by a mutation in a gene that makes the oxygen-carrying protein in blood. After modifying those cells to make a normal version of the protein, called hemoglobin, they’ll give the cells back to the patient and reform the blood system with normal, healthy blood.

Manipulating the Numbers

There’s more than one way to modify a stem cell. Although the HIV/AIDS and sickle cell teams rely on genetic modifications, a team lead by Eduardo Marban at Cedars-Sinai in Los Angeles is manipulating the numbers. His team plans to attack heart failure by harvesting tidbits of heart muscle and multiplying the regenerative cells within to much higher numbers than naturally occur in the heart. In four weeks, some 25 million stem cells will be available to inject directly into the muscle of a failing heart using special needle injection catheters. With the successful completion of animal trials, Marban hopes to start studies in patients with severe heart failure in about two years.

"Even though we've gotten better at treating heart failure, when patients are sufficiently afflicted -- they have shortness of breath and decreased ability to exercise -- the mortality for this disorder exceeds that of many malignant tumors," he said.

Although heart transplantation can save lives, there are only 2,000 donor hearts available every year, and nearly 100,000 people a year who need them.

"Stem cell-based treatment could be a cellular transplantation alternative to organ transplant," Marban said.

Next: Embryonic Cells

Although researchers today are testing the patient’s own cells, ideally, embryonic stem cells will replace patient cells, said Chen, who leads the UCLA HIV/AIDS disease team. “The advantage of embryonic stem cells is that you can grow and modify them with desired genetic traits and stockpile them," he said. "When somebody needs a transplant, we’ll pull out a vial (with an immune profile that matches the patient’s) and transplant the patient. That would be ideal, but we’re not there yet.” Adult stem cells were discovered ten years before their more flexible embryonic counterparts, thus their head start in early therapies.

A disease team lead by Albert Lane at Stanford University has made the transition from adult to embryonic-like cells. They intend to reprogram skin cells from people with a rare skin disease called dystrophic epidermolysis bullosa and reprogram those into so-called iPS cells, which mimic embryonic stem cells in many ways. After repairing the mutation that causes the disease, they’ll mature those

cells into skin and use the resulting cellular sheets to replace the patient's own damaged skin.

According to Chen, who leads the UCLA HIV/AIDS disease team, all of these projects — whether they are in blood, brain or skin or any other organ —are of growing importance to HIV patients. That's because advances in any of the projects mean a better understanding of how to tweak stem cells for therapeutic benefit. Tweaks that could be put to use in treating HIV/AIDS.

"These advances are beneficial to HIV," Chen said. If one team discovers better ways of modifying stem cells, it could help knock out CCR5 in people with HIV. "If we advance in cardiovascular disease, we'll help people with HIV. A significant advance against cancer makes a difference for HIV. It's all significant. It makes a difference."

Neural Assassins

Some of those anticipated advances may well come from glioma disease teams, based on work with neural stem cells started all those years ago by Snyder and Aboody, The two glioma disease teams propose the use of something called a pro-drug, a Jekyll-and-Hyde cancer-killer that requires a chemical signal to convert it from meek to mean. Neural stem cell, beckoned toward the tumor cells, will be engineered to release the chemical signal that turns the pro-drug into the killer.

Aboody plans to use a pro-drug called CPT-11, also known as irinotecan, as a form of chemotherapy. On it's own, CPT-11 kills nothing, but in the presence of the enzyme carboxylesterase, also called CE, it turns into the very potent chemotherapeutic agent called SN-38.

"SN-38 is way too toxic to administer," Aboody said. Doctors use CPT-11 in liver and colon cancer, where naturally occurring CE converts it to the more powerful drug. But the brain produces no CE. That's where the stem cells come in.

Mouse studies suggest that they are on to something. Stem cells engineered to secrete CE were put into the brain of mice with human brain tumor. Four days later — when all stem cells were in place — the mice received CPT-11 and the tumor cells were killed.

In other research, Aboody demonstrated the neural stem cell's ability to target and kill a variety of cancers, including melanoma brain metastases, the brain cancer medullablastoma, and metastases outside the brain from the childhood cancer metastatic neuroblastoma. Others have shown stem cells localize to metastatic breast cancer lesions in the brain as well.

The stem cells "are not drawn to every cancer," Aboody said, but studies so far suggest, "The more aggressive the cancer, the more the attraction."

Those are results that would have surprised the young Evan Snyder, talking to preoccupied geneticists, as he first laid out his thoughts for this field that is becoming such a rich source of future therapies.
